IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

GARY M. FADER

CASE NO.: BB1071USDIV2 — APPLICATION NO.: 10/757667

CONFIRMATION NO.: 7292 — GROUP ART UNIT: 1638

EXAMINER: VINOD KUMAR — FILED: JANUARY 14, 2004

FOR: SUPPRESSSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Via EFS-Web

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Brief on Appeal

This is an appeal of the Final Rejection, mailed July 16, 2008, of claims 1, 12, 16 and 26 of the above-identified application.

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(I) Real Party in Interest

The real party in interest in this Appeal is E. I. du Pont de Nemours and Company, the assignee of the entire right, title and interest of the above-identified patent application.

(II) Related Appeals and Interferences

There are no related Appeals or Interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending Appeal.

(III) Status of Claims

Claims 1-21 were originally filed.

Claims 22-24 were entered by Preliminary Amendment, claims 2-21 were cancelled and claim 1 was retained for purposes of continuity and it was cancelled upon entry of the Preliminary Amendment.

There are three independent claims: 22, 23 and 24.

The currently pending and appealed claims are claims 22, 23 and 24 which are set forth in the Claims Appendix attached hereto.

(IV) Status of Amendments Filed Subsequent to Final Rejection

A Response after Final was filed electronically on December 18, 2008 and was entered for purpose of this Appeal according to the Advisory Action electronically delivered on February 9, 2009.

(V) Summary of the Invention

The invention on appeal concerns food comprising a soy protein product prepared from transgenic soybeans seeds having a reduced quantity of soybean seed storage protein prepared by methods recited in the claims and, also, that constitute the subject matter of U.S. Patent No. 6,703,544 issued on March 9, 2004 to Fader et al.

Claim 22 recites food comprising a soy protein product prepared from transgenic soybean seeds having a reduced quantity of soybean seed storage protein and prepared by a method comprising:

(a) constructing a chimeric gene comprising:

   (i) a nucleic acid fragment comprising a promoter that is functional in the cells of soybean seeds;

   (ii) a nucleic acid fragment encoding all or a portion of a soybean seed storage protein placed in sense or antisense orientation relative to the promoter of (i) wherein said soybean seed storage protein is selected from the group consisting of glycinin and β-conglycinin; and

   (iii) a transcriptional termination region;

(b) creating a transgenic soybean cell by introducing into a soybean cell the chimeric gene of (a); and

(c) growing the transgenic soybean cells of step (b) which express the chimeric gene of step (a)

wherein the quantity of one or more members of a class of soybean seed storage protein subunits is reduced when compared to soybean seeds not comprising the chimeric gene of step (a), and wherein the class of soybean seed storage protein subunits is selected from the group consisting of: glycinin and β-conglycinin.

Support for this claim can be found on pages 1 and 2 and elsewhere in the specification, in particular, the examples and claims as originally filed.

Claim 23 is similar to Claim 22 except that food comprises a soy protein product prepared from transgenic soybean seeds prepared by a method for simultaneously reducing expression of two soybean genes. Support for this claim can be found on pages 1-2 of the specification, and elsewhere in the specification, in particular, the examples and claims as originally filed.

Claim 24 recites food comprising a soy protein prepared from transgenic soybean seeds obtained from a soybean plant transformed at a single locus in its

genome with a chimeric gene comprising at least a portion of a glycinin or a β-conglycinin gene for reducing the amount of at least one soybean seed storage protein in soybean seeds wherein the seed storage protein is selected from the group consisting of glycinin and β-conglycinin, when compared to seeds obtained from a soybean plant not comprising the chimeric gene in its genome.

Support for this claim can be found on pages 1-2 of the specification, in Example 2 and further data relating to Example 2 was set forth in the declaration of Dr. Anthony Kinney, one of the co-inventors of the subject application. A copy of Dr. Kinney's declaration dated June 29, 2001 is set forth in Evidence Appendix A. Also included in Evidence Appendix B is a copy of Dr. Gary Fader's declaration dated June 27, 2001. Dr. Fader's declaration shows that all the glycinin subunits were suppressed when truncated form of the G1 and G4 subunits were expressed in a sense orientation under the control of a Kti promoter.

(VI) Grounds of Rejection To Be Reviewed on Appeal

There are two grounds of rejection presented for review:

a) Whether claims 22, 23 and 24 are anticipated under 35 USC §102(b) or, in the alternative, are rendered obvious under 35 USC §103(a) over Trueblood et al. (U.S. Patent No. 4,267,118 issued on May 12, 1981?

b) Whether claims 22, 23 and 24 are anticipated under 35 USC §102(b) or, in the alternative, are rendered obvious under 35 USC §103(a) over Staswick et al. (Archives of Biochemistry and Biophysics, 223:1-8 1983)?

(VII) Argument

(a) The rejection of claims 22, 23 and 24 as anticipated under 35 USC §102(b) or, in the alternative, as rendered obvious under 35 USC §103(a) over Trueblood et al. (U.S. Patent No. 4,267,118 issued on May 12, 1981.

Claims 22-23 concern food comprising a soy protein prepared from transgenic soybean seeds prepared according to the methods recited therein.

It is alleged that the claimed food has the same structural limitations as taught by Trueblood et al. The '118 patent (Trueblood et al.) concerns a process for treating crude soybean oil to make it a food or commercial grade quality. It is stated in column 4 at lines 41 to 48 that

> the **protein level** in the supernatant oil or that being separate from the treatment vessels as above described, was found to be **less than 0.1 gram protein per 100 grams of oil (0.1%)**. A protein analysis of the crude soybean oil prior to treatment in accordance with the present invention showed a level of 1.5 gram protein per 100 grams of oil (1.5%). (Emphasis added.)

Thus, the method of Trueblood et al. is to produce a food grade quality soybean oil from crude soybean oil wherein the resulting food grade quality soybean oil has a protein content of less than 0.1%.

Submitted herewith in Evidence Appendix C is a copy of a portion of Soy Protein Products: Characteristics, Nutritional Aspects and Utilization published by the Soy Protein Council (1987). It is stated on page 1 of this reference that

> Soy protein products fall into three major groups. These groups are based on protein content, and range from 40% to over 90%. All three basic soy protein product groups (except full-fat flours) are derived from defatted flakes. They are: soy flours and grits, soy protein concentrates and soy protein isolates (Table 1). . . .

It is clear that, by definition, the lowest level of soy protein in a soy protein product is about 40% protein. Clearly, a food grade soybean oil having less than 0.1% protein does not constitute a soy protein product by even the wildest stretch of the imagination and constitutes merely an impurity.

Also, submitted herewith in Evidence Appendix D is a printout from the soy foods web page, www.soyfoods.org. This also shows that a soy protein product such as soy flour would have at least 40% protein.

Accordingly, one of ordinary skill in the art is inexorably led to the conclusion that a soy protein product would have at least about 40% protein and

that a food-grade quality oil having less than 0.1% protein does not constitute a soy protein product as that term is defined as set forth in the aforementioned publication by the Soy Protein Counsel.

The contention that the food of the instant invention comprising a soybean protein product prepared from transgenic soybeans seeds having a reduced quantity of soybean seed storage protein prepared by methods recited in the claims is anticipated by a food grade soybean oil having less than 0.1% protein as described by Trueblood et al. is utterly without merit.

Those skilled in the art know that a soybean protein product, by definition, has a protein content of at least 40% as supported by the discussion of the references in Evidence Appendices C and D. A food grade soybean oil having less that 0.1% protein does not, even remotely, come close to minimum level of about 40% protein that characterizes a soybean protein product.

b) The rejection of claims 22, 23 and 24 as anticipated under 35 USC §102(b) or, in the alternative, are rendered obvious under 35 USC §103(a) over Staswick et al. (Archives of Biochemistry and Biophysics, 223:1-8 1983).

Staswick et al. are concerned with improving the nutritional quality of soybean seed protein by altering glycinin subunit composition. The cultivar used in the in study does not appear to be transgenic. The focus of Staswick et al. is in improving the nutritional quality of glycinin storage protein by replacing subunits having a low methionine content with those having a higher methionine content.

In contrast, the instant invention concerns food comprising a soy protein product prepared from transgenic soybean seeds having a reduced quantity of soybean seed storage protein ....wherein the quantity of one or more members of a class of soybean seed storage protein subunits is reduced when compared to soybean seeds not comprising the **chimeric gene** (emphasis

added) of step (a), and wherein the class of soybean seed storage protein subunits is selected from the group consisting of glycinin and β-conglycinin.

"***Chimeric gene***" (emphasis added) is defined in the specification on page 8, lines 17-28 *"as a gene that comprises heterogeneous regulatory and coding sequences not found in nature.*"

It is stated on page 6 of the Office Action dated October 15, 2008 that "...it is noted that the features upon which applicant relies (i.e., soy protein products as claimed contains chimeric construct) are not recited in the rejected claim(s). . . ."

Such features are, at a minimum, inherently present in the claimed invention .

Food of the instant invention comprises a soy protein product prepared from transgenic soybean seeds having a reduced quantity of soybean seed storage protein ....wherein the quantity of one or more members of a class of soybean seed storage protein subunits is reduced when **compared to soybean seeds not comprising the chimeric gene** (emphasis added) of step (a), and wherein the class of soybean seed storage protein subunits is selected from the group consisting of: glycinin and β-conglycinin.

Accordingly, food of the instant invention comprising a soy protein product as recited in the claims would be distinguishable by the presence of the chimeric gene used to create the transgenic soybean plant producing the seeds from which the soy protein products were obtained.

Methods for detection of chimeric genes in biological and in food material a very well known in the art and performed on a routine basis by those skilled in the art.

<u>(VIII) Conclusion</u>

In view of the foregoing discussion, it is respectfully submitted that:

a) claims 22-24 are neither anticipated under 35 USC §102(b) nor rendered obvious under 35 USC §103(a) over Trueblood et al.; and

b) claims 22-24 under 35 USC §102(b) are not anticipated by, or in the alternative, under 35 USC §103(a) obvious over Staswick et al

Accordingly, the Board is respectfully requested to reverse the final rejection of pending claims 1, 12, 16 and 26 and indicate allowability of all claims.

Enclosed herewith is a Petition for a one (1) month extension of time to permit the filing of the Brief on Appeal. Please charge the fee for extension of time of one (1) month, as well as the requisite fee set forth in 37 CFR §1.17(f), to Appellant's Assignee's (E. I. du Pont de Nemours and Company) Deposit Account No. 04-1928.

Respectfully submitted,

/Lynne M. Christenbury/

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Dated: March 16, 2009

Claims Appendix

Claim 22. (previously presented) Food comprising a soy protein product prepared from transgenic soybean seeds having a reduced quantity of soybean seed storage protein and prepared by a method comprising:

(a) constructing a chimeric gene comprising:

(i) a nucleic acid fragment comprising a promoter that is functional in the cells of soybean seeds;

(ii) a nucleic acid fragment encoding all or a portion of a soybean seed storage protein placed in sense or antisense orientation relative to the promoter of (i) wherein said soybean seed storage protein is selected from the group consisting of glycinin and β-conglycinin; and

(iii) a transcriptional termination region;

(b) creating a transgenic soybean cell by introducing into a soybean cell the chimeric gene of (a); and

(c) growing the transgenic soybean cells of step (b) which express the chimeric gene of step (a)

wherein the quantity of one or more members of a class of soybean seed storage protein subunits is reduced when compared to soybean seeds not comprising the chimeric gene of step (a), and wherein the class of soybean seed storage protein subunits is selected from the group consisting of: glycinin and β-conglycinin.

Claim 23. (previously presented) Food comprising a soy protein prepared from transgenic soybean seeds prepared by a method for simultaneously reducing the expression of two soybean genes comprising:

(a) constructing a chimeric gene comprising:

(i) a nucleic acid fragment comprising a promoter region from a soybean seed storage protein gene; and

(ii) a nucleic acid fragment encoding all or a portion of a soybean protein that is not the soybean seed storage protein of (i) wherein said soybean seed storage protein is selected from the group consisting of glycinin and β-conglycinin, said nucleic acid fragment placed in a sense or antisense orientation relative to the promoter of (i), and (iii) a transcriptional termination region;

(b) creating a transgenic soybean seed by introducing into a soybean seed the chimeric gene of (a); and

(c) growing the transgenic soybean seeds of step (b) which express the chimeric gene of step (a);

wherein the quantity of one or more members of a class of soybean seed storage protein subunits and the quantity of the protein encoded by the nucleic acid fragment of (a)(ii) is reduced when compared to soybeans seeds not comprising the chimeric gene of step (a), and wherein the class of soybean seed storage protein subunits is selected from the group consisting of glycinin and β-conglycinin.

Claim 24. (previously presented) Food comprising a soy protein prepared from transgenic soybean seeds obtained from a soybean plant transformed at a single locus in its genome with a chimeric gene comprising at least a portion of a glycinin or a β-conglycin gene for reducing the amount of at least one soybean seed storage protein in soybean seeds wherein the seed storage protein is selected from the group consisting of glycinin and β-conglycinin, when compared to seeds obtained from a soybean plant not comprising the chimeric gene in its genome.

EVIDENCE APPENDIX A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

ANTHONY J. KINNEY GARY M. FADER	CASE NO.: BB-1071-A
APPLN. NO.: 09/108,010	GROUP ART UNIT: 1638
FILED: JUNE 30, 1998	EXAMINER: E. MCELWAIN

FOR: SUPPRESSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Anthony J. Kinney Pursuant to 37 CFR §1.132

I am one of the above-identified inventors named in this application. I, Anthony John Kinney, am a citizen of the United Kingdom and am a permanent resident of the United States of America, residing at 609 Lore Avenue, Wilmington, Delaware 19809, and I declare as follows:

I received a B.Sc. in biology from the University of Sussex in 1980 and a D. Phil. in biochemistry and cell biology from Oxford University in 1985. I served as a research fellow in the Department of Food Science at Rutgers University, New Brunswick, N.J. 9/87-5/89. I have been employed at E. I. du Pont de Nemours and Company (DuPont) since June, 1989. I work as a technical leader for DuPont Crop Genetics and am presently working on expression of storage oil, protein and, carbohydrate genes. I have authored in excess of fifteen refereed articles in the field of biochemistry, with emphasis in the field of fatty acid and oil biosynthesis.

2. I have reviewed the Office Action dated March 23, 2001. I am aware that this declaration is being submitted to address the concerns set forth on pages 4 and 5 of the Office Action that "even though the product by process claims are limited and defined by a process, the determination of patentability is based on the product itself."

3. The results shown in the application indicate that the transgene responsible for the phenotype was integrated into a single locus. Example 2 of the application, in particular page 24 at lines 4 through 13, describes the isolation of transgenic soybean

sublines (G94-1, G94-19) with high oleic acid and suppressed β-conglycinin subunits derived from transformation event 260-05. The sublines are described as containing two copies of the plasmid pBS43 at a ***single*** locus, called the "transwitch locus" in example 2 and *locus A* in this declaration. *Locus* A is responsible for both the high oleic and the β-conglycinin null phenotype. Example 2 also states that R5 seeds derived from both of these sublines (G94-1, G94-19) have suppressed β-conglycinin subunits. (For clarity it should be noted that the terms "Transwitch locus" and "*locus A*" are used interchangebly herein.)

4. The Southern blot analyses presented below further analyze the transgenic seeds and plants and show that the phenotype is due to the presence of an insertion at a single locus. The experiments discussed herein were performed by me or others working under my guidance and direction or in coordination with the DuPont regulatory group.

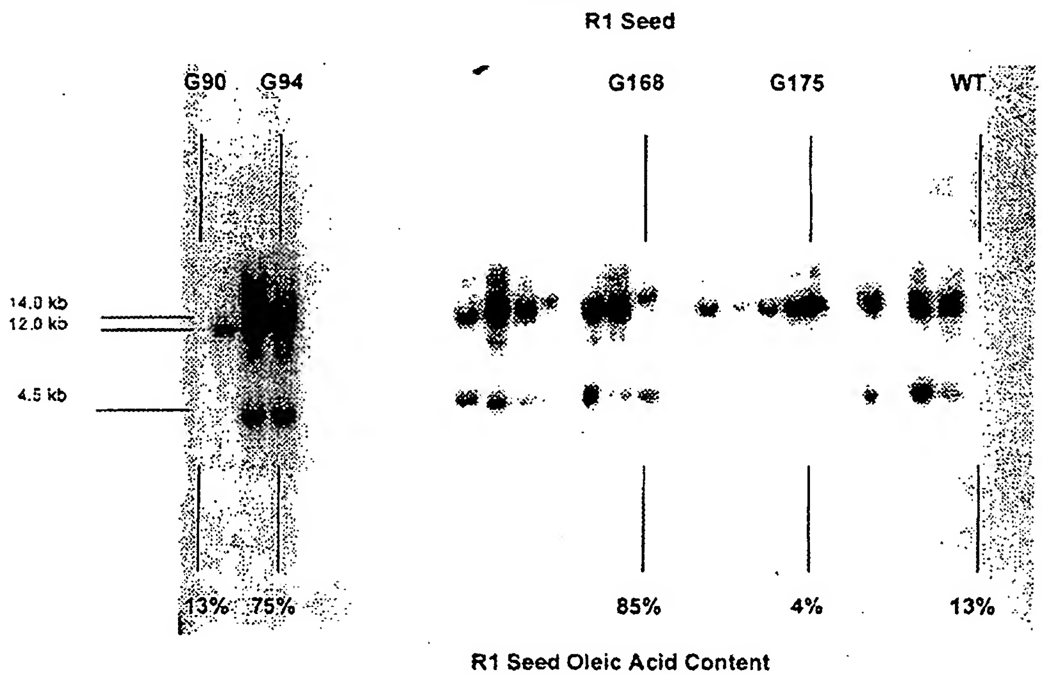
5. The nature of the insert at *locus A* ("transwitch locus") was initially determined by Southern blot analyses of DNA from leaves of R1 and R2 plants. Genomic DNA was digested with Bam HI and probed with the 3' region of phaseolin to detect the *GmFad 2-1* gene expression cassette. Bam HI cuts once in the plasmid and would be expected to result in one hybridising band for each copy of the plasmid inserted into the genome. The results of Southern blot analysis of DNA isolated from leaf tissue of event 260-05 R1 plants that were grown from chipped seeds analysed for fatty acid composition are shown below in Figure 1.

The DNA hybridisation pattern depicted in Figure 1 shows clearly that in the original transformation event the *GmFad 2-1* construct was integrated at two different loci in the soybean genome. At *locus A* the *GmFad 2-1* construct silenced the endogenous *GmFad 2-1* gene, resulting in seeds with an oleic acid content generally above 80%. *Locus A* contained two copies of the *GmFad 2-1* expression cassette as indicated by the two hybridising fragments of 14.0 kb and 4.5 kb. The second locus (*locus B*) contained a copy of *GmFad 2-1* that was over-expressing thus decreasing oleic acid levels to around 4%. *Locus B* contained only one copy of the *GmFad 2-1* expression cassette as noted by the single hybridising fragment of 12.0 kb.

Since G94 contained both loci in the R1 plant an additional round of selection was necessary on the segregating R2 plants to isolate plants containing *locus A* and not *locus B*. Southern blot analysis on genomic DNA isolated from leaf tissue of R2 plants grown from G94 R2 seed using Bam HI digestion and the phaseolin 3' probe identified two sublines, G94-1 and G94-19, that contained *locus A* but not *locus B* since *locus B* had been removed by segregation. *Locus B* was not further

characterized. Figure 2 shows Southern blot analysis of R1 and R2 leaf tissue originating from 260-05 G94 R1 seed. The genomic DNA was digested with Bam HI and probed with the phaseolin 3' probe to detect the integration of the GmFad 2-1 construct.

Figure 1



Additional Southern blot analyses using DNA from the leaves of R6 plants, using multiple restriction enzymes, confirmed that G94-1 and G94-19 contained a single transgenic locus (*locus A*, the "Transwitch locus"). Figure 3 shows the results of Southern blot analyses of DNA isolated from R6 leaf tissue of G94-1 and G94-19, of a sister line from the same event (G168), and of control elite soybean line A2396. In this figure DNA was digested with either Bam HI, Bsp HI, Hind III, or Sst I and hybridized with the phaseolin 3' probe. The Bam HI pattern is identical to the R2 plants.

7. The results mentioned in the application and expanded upon above indicate that suppression of β-conglycinin subunits is caused by an insertion in a single locus of the transgenic plant.

Figure 2

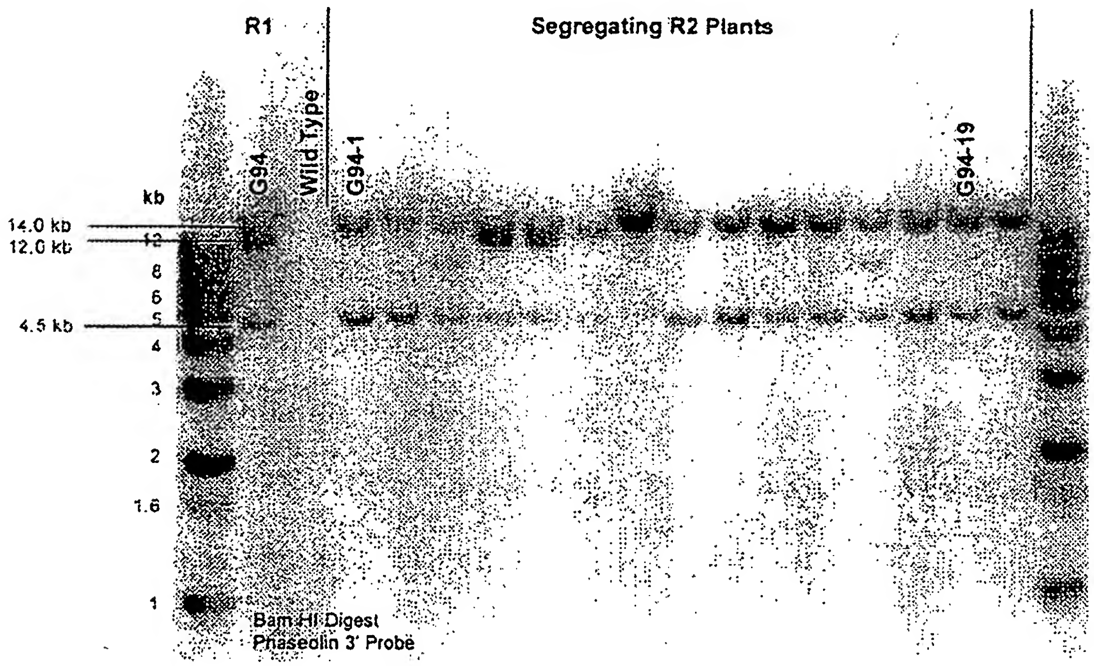
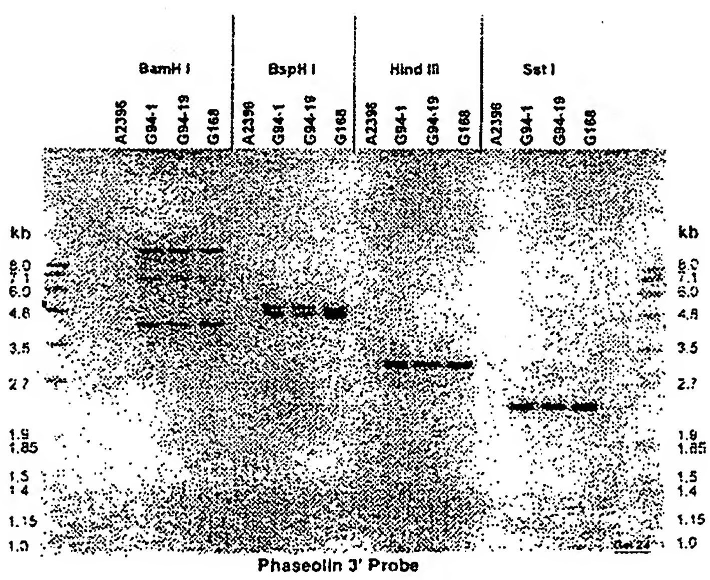
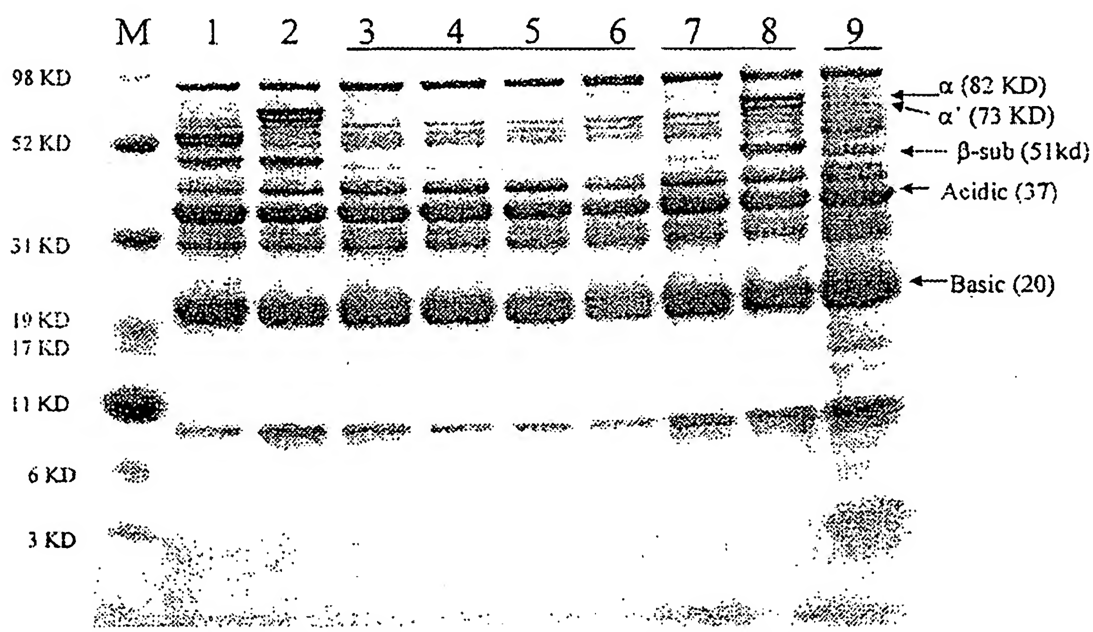


Figure 3



8. Furthermore, analysis, as described in Example 3, of other transgenic soybean lines containing exactly the same α'-subunit promoter sequence of the construct in Example 2 indicates that R1 seeds of these events lack the α, α', and sometimes β subunits of β-conglycinin. Figure 4 shows a picture of a Coomassie brilliant blue R-stained SDS polyacrylamide gel where 20 μg total protein was loaded per lane. The origin of the material loaded on each lane is indicated below the picture of the gel.Figure 4

Figure 4



1. 260-05(G94-1)(85%18:1)
2. JACK (20%)
3. 2308-22-16-H5 (80%)
4. 2308-22-3-14 (80%)
5. 2308-23-5-H1 (72%)
6. 2308-23-6-H13 (72%)
7. 2323-31-1-H34(82%)
8. 2323-31-10-9 (82%)
9. 2335-26-14-11 (80%)
10. 2335-26-2-8 (80%)

In summary, all of the elements of the claimed invention were provided in the patent application. The data presented in this declaration are consistent with the disclosure set forth in the specification.

Accordingly, one skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Anthony J. Kinney

29 JUNE 2001

Date

EVIDENCE APPENDIX B

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

ANTHONY J. KINNEY
GARY M. FADER

CASE NO.: BB-1071-A

APPLN. NO.: 09/108,010

GROUP ART UNIT: 1638

FILED: JUNE 30, 1998

EXAMINER: E. MCELWAIN

FOR: SUPPRESSION OF SPECIFIC CLASSES OF SOYBEAN SEED PROTEIN GENES

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Gary Fader Pursuant to 37 CFR §1.132

I, Gary M. Fader, am a citizen of the United States of America, residing at 100 Woods Lane, Landenberg, PA 19350, United States of America, and I declare as follows:

1. I am one of the above-identified inventors named in this application. I am a graduate of the University of Toledo, Ohio with a B.A. degree granted in 1979 in Biology. I received an M.S. in Crop Physiology in 1981 and a Ph.D. in Crop Physiology in 1983 from Purdue University. I was a postdoctoral fellow at the Agronomy Department of the University of Wisconsin from 1983 to 1986. I have been employed by E. I. du Pont de Nemours and Company since 1986 directing and conducting research in developing herbicide resistant plant varieties and developing soybean lines with improved oil and protein qualities. I have built small-scale processing capabilities to produce oils and protein products for evaluation, developed small-scale functional tests predictive of performance in food applications, germplasm screening, manipulation of gene expression using molecular biology, and transformation and regeneration of plants.

2. I have reviewed the Office Action dated March 23, 2001. I am aware that this declaration is being submitted to address the concerns set forth on pages 2 and 3 of the Office Action that "the claims are broadly drawn to the use of an unspecified gene to produce the claimed plants and seeds with reduced levels of glycinin of β-conglycinin, yet the specification only teaches the use of one particular gene to produce said plants and seeds."

3. The rationale for combining the nucleic acid fragments of the invention clearly was disclosed in the specification. It was shown, for the first time, that two or more subunits of β-conglycinin could be suppressed using:

a) a truncated alpha subunit of β-conglycinin in sense orientation with respect to a promoter, or

b) β-conglycinin promoter and leader sequences directing the expression of sense FAD2, or

c) the entire alpha subunit coding region in anti sense orientation with respect to a promoter.

The specification also disclosed that expression of truncated glycinin subunits would suppress glycinin (all subunits).

4. Methods to prepare DNA fragments comprising truncated versions of the different glycinin subunits were set forth in the specification. The specification also described how to use these nucleic acid fragments to practice the invention.

5. The fragments corresponding to the glycinin Group I (G1) and Group II (G4) described in Example 4 of the specification (page 26 at line 3 through page 27 at line 31) were joined in a transcription unit under the control of the KTi promoter and used for bombardment into somatic embryo tissue. The transcription unit containing KTi promoter/G1/G4/KTi 3' end was cloned into the Bam HI site of pKS18HH. Plasmid pKS18HH is described in the application on page 15 at line 40 through page 16 at line 3 and is shown in the application's Figure 3. The plasmid used for bombardment contained:

a) the KTi promoter/G1/G4/KTi 3' end

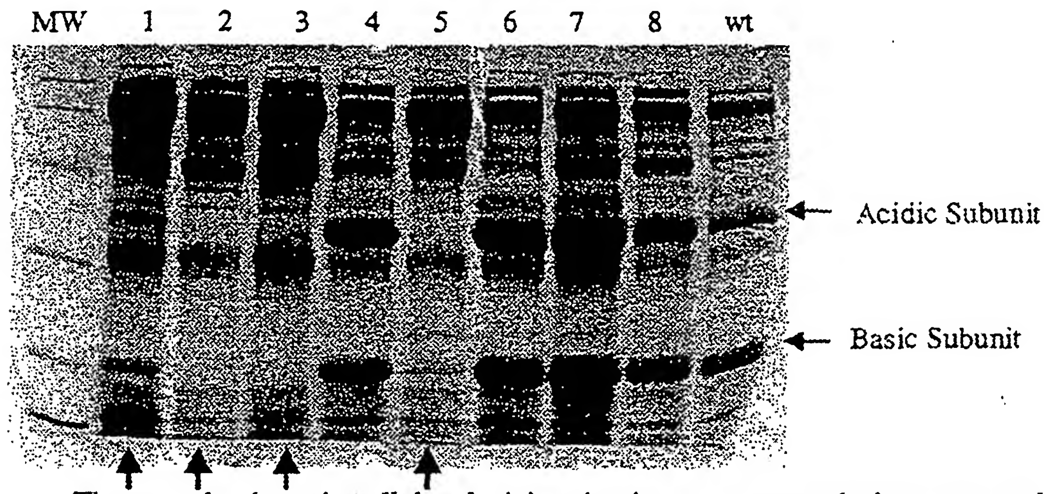
b) the T7 promoter/HPT/T7 Terminator Sequence

c) the CaMV 35S promoter/HPT/NOS 3' end

d) the vector sequences from pSP72 with the beta-lactamase coding region removed.

Bombardment and analyses were conducted as described in the specification on page 17 at line 10 through page 18 at line 37.

6. The results (an example of which is shown in the SDS PAGE gel of protein extracted from seeds of lines derived from regenerated plants) indicate that all the glycinin subunits are suppressed in some of the lines (indicated by arrows at the bottom of the gel).



These results show that all the glycinin subunits are suppressed when truncated forms of the G1 and G4 subunits are expressed in sense orientation under the control of the KTi promoter.

In summary, all of the elements of the claimed invention were provided in the patent application. The data presented in this declaration are consistent with the disclosure set forth in the specification.

Accordingly, one skilled in the art can take these elements, as discussed above, and practice the invention without undue experimentation.

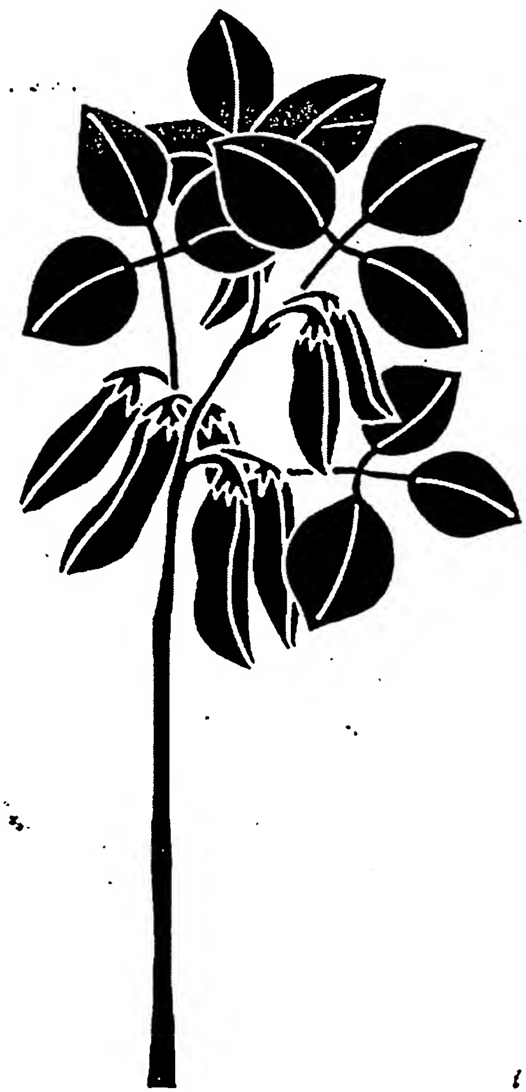
I declare further that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Gary M. Fader

20 6-27-01
Date

Soy Protein Products

Characteristics, Nutritional Aspects and Utilization



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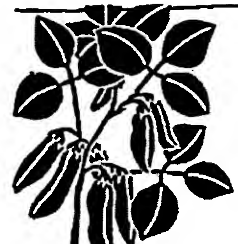


II. Definitions And Methods Of Preparation

The soybean plant (*Glycine max*) belongs to the legume family. It is able to utilize the nitrogen of the air through the action of bacteria on its roots. The protein content of the seed is about 40%. After the hulls and the oil are removed, the remaining defatted flake, which is the starting material for most commercial protein ingredients, has a protein content of approximately 50%.

Soybeans entering the processing plant are screened to remove damaged beans and foreign materials, then treated as shown in Figure 1. The oil is removed from the flakes by a solvent (hexane) in one of several types of countercurrent extraction systems. After the defatted flakes leave the extractor, any residual solvent is removed by heat and vacuum.

Soy protein products fall into three major groups. These groups are based on protein content, and range from 40% to over 90%. All three basic soy protein product groups (except full-fat flours) are derived from defatted flakes. They are: soy flours and grits, soy protein concentrates and soy protein isolates (Table 1).

There are also specialty products based on traditional Oriental processes, which utilize the entire bean as starting material.

Soy Flours and Grits

Soy flours and grits are made by grinding and screening soybean flakes either before or after removal of the oil. Their protein content is in the range of 40% to 54%.

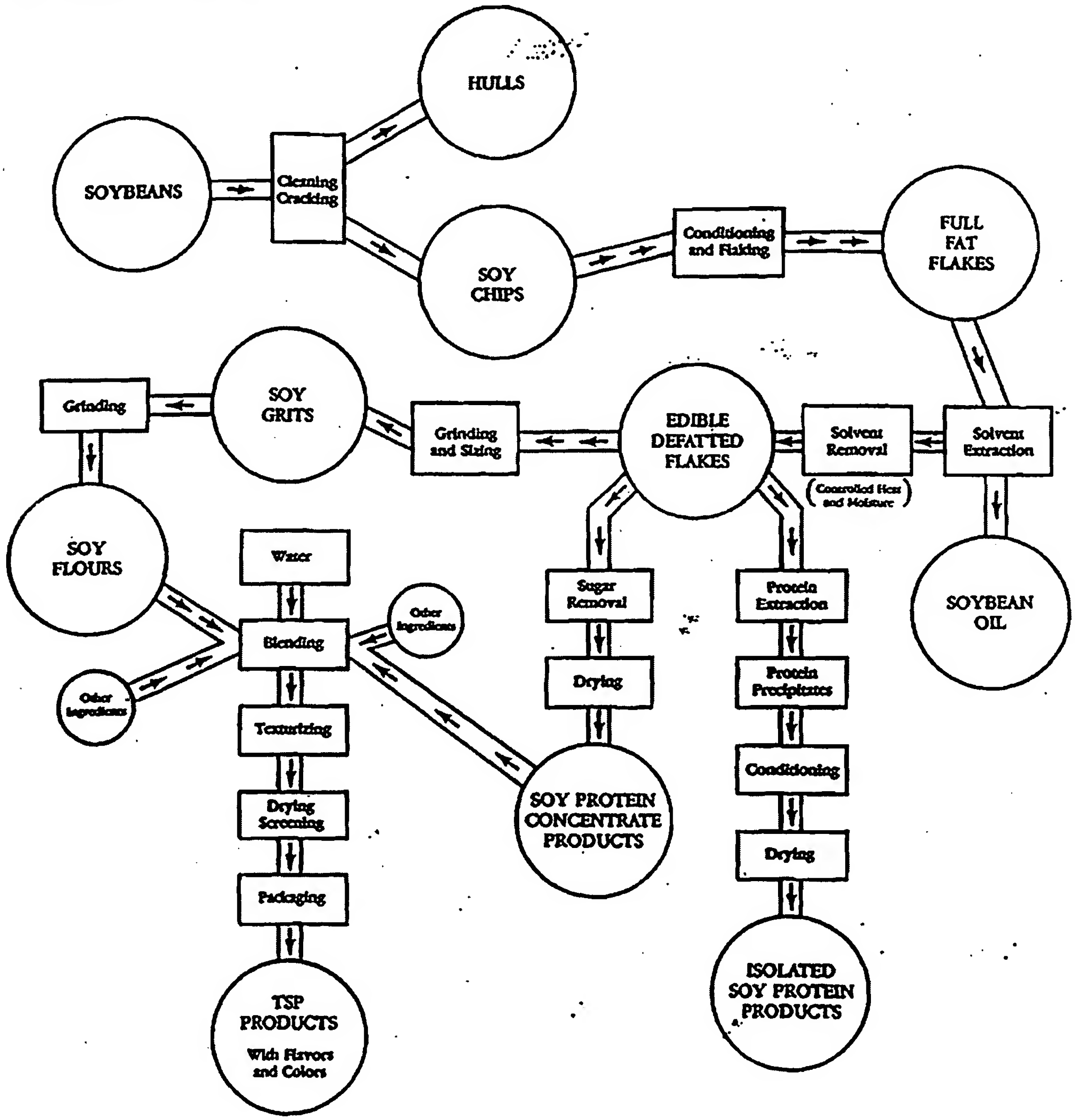
Soy flours and grits are the least refined forms of soy protein products used for human consumption and may vary in fat content, particle size, and degree of heat treatment. They are also produced in lec thinated or refatted forms. The degree of heat trea ment creates varying levels of water dispersibilit a quality that can be useful in tailoring functionalit in many food applications. Preparation and uses c various flours are as follows:

Type	Preparation	Uses
Full-fat flours (40% protein*)	Dehulled cotyledons are milled to specific size.	Produced primarily in Europe and Asia for the baking indus try and the production of soy milks.
High enzyme flours (52% to 54% protein*)	Produced from defatted flakes with minimum heat. High NSI**	Increasing mixing tolerance and bleach ing in bread; prepar tion of functional concentrates and isolates.
Defatted flours (52% to 54% protein*)	Finely ground to pass through a No. 100 U.S. Standard Screen size. Controlled moist heat treatment used to provide 'white' (NSI 85 to 90), 'cooked' (NSI 20 to 60), and 'toasted' (NSI below 20) grades.	Varied uses requirin a wide range of pro tein solubilities.
Defatted grits (52% to 54% protein)	Screen size between No. 10 and 80. Otherwise the same as flours.	Ground meat syster and bakery produc
Lecithinated/ Refatted flours	Lecithin or vegetable oil is combined with defatted flakes (0.5% to 30%).	Improving water dispersibility and emulsifying capabil in baking applications.

(*) N×6.25
(**) Nitrogen Solubility Index (NSI), as is basis

Figure 1.

SOY PROTEIN PROCESSING



mfb: moisture free basis

TABLE 1.
COMPOSITION OF SOY PROTEIN PRODUCTS

	Defatted Flours & Grits		Concentrates		Isolates	
Constituent	as is	mfb*	as is	mfb*	as is	mfb'
Protein (N×6.25)	52–54	56–59	62–69	65–72	86–87	90–9
Fat (pet. ether)	0.5–1.0	0.5–1.1	0.5–1.0	0.5–1.0	0.5–1.0	0.5–1
Crude Fiber	2.5–3.5	2.7–3.8	3.4–4.8	3.5–5.0	0.1–0.2	0.1–0
Ash	5.0–6.0	5.4–6.5	3.8–6.2	4.0–6.5	3.8–4.8	4.0–5
Moisture	6%–8%	0	4%–6%	0	4%—6%	0
Carbohydrates (by Difference)	30–32	32–34	19–21	20–22	3–4	3–4

*mfb: moisture free basis

Soy Protein Concentrates

Soy protein concentrates are prepared from dehulled and defatted soybeans by removing most of the water-soluble, non-protein constituents as summarized in the following column. They contain at least 65% protein (N×6.25) on a moisture-free basis (mfb).

Type	Preparation	Uses
Soy Protein Concentrates	Produced by three basic processes: acid leaching (at about pH 4.5), extraction with aqueous alcohol (70% to 90%), and denaturing the protein with moist heat prior to extraction with water.	Varied applications requiring low-flavor profile, water and fat absorption, and emulsification (dispersible form). Nutritional applications.

Neutralized concentrates prepared by acid leaching have a higher water-soluble protein content than those prepared by either alcohol leaching or heat denaturation techniques. In a more recently developed process, a low water-soluble soy protein co centrate (aqueous alcohol extraction) is subjectec heat treatment by steam injection or jet cooking increase solubility and functionality. Functional may be improved further by additional treatmen a homogenizer. These concentrates function as en sifiers and emulsion stabilizers, they bind fat a water, and they offer special adhesive propert similar to those of isolates.

Soy Protein Isolat

Isolates are the most highly refined soy prot products commercially available. They represent major proteinaceous fraction of the soybean. ! isolates are prepared from dehulled and defatted s beans by removing most of the non-protein co ponents as summarized in the accompanying ch They contain not less than 90% protein (N×6. on a moisture-free basis.

Isolates may also be lecithinated to improve dispersibility and to reduce dusting. Both gelling non-gelling varieties are available, as well as vary grades of viscosity.

Type	Preparation	Uses
Soy Protein Isolates (Isoelectric and Neutralized)	The protein is extracted from defatted soybean flakes with water or mild alkali in a pH range of 8 to 9 followed by centrifuging to remove insoluble fibrous residue; adjusting resulting extract to pH 4.5 where most of the protein precipitates as a curd; separating curd by centrifugation from the soluble oligosaccharides, followed by multiple washings, and then spray-drying to yield an 'isoelectric' isolate.	Infant formulas and nutritional applications.
	More commonly, the isolate is neutralized (Na or K proteinates) to make it more soluble and functional. About one third of starting flake weight is recovered in the form of an isolate.	Meat and dairy products. Varied applications requiring emulsification/emulsion stabilization; water and fat absorption; adhesive/fiber forming properties. Food analogs.

Textured Soy Proteins

Textured soy proteins (TSP) are processed to impart a structure, such as fiber or chunk, for use as a food ingredient. They are frequently made to resemble meat, seafood or poultry in structure and appearance when hydrated. Their preparation and uses are as follows:

Type	Preparation	Uses
Textured Flours and Concentrates	Thermoplastic extrusion or steam texturization of soy flours or alcohol/heat denatured concentrates. Composition is similar to the corresponding source material.	Many types of fibrous foods, ground meat products, poultry and seafoods.
Structured Concentrates	Processing through an extruder into different sizes and shapes.	Poultry, meats and seafoods.
Structured Isolates	Extrusion as above or by extruding a solution of the isolate into an acid-salt bath that coagulates the protein into fibers that are combined with binders to form fiber bundles.	Poultry and seafoods. Food analogs.

Specialty Soy Foods and Ingredients

Partially hydrolyzed soy protein products are products obtained by cleavage of the protein by proteolytic enzymes, such as pepsin, papain, and bromelain to reduce the molecular weights of proteins to a range of 3,000 to 5,000. This improves whipping properties and acid solubility.

Fully hydrolyzed proteins used as flavoring agents can be prepared from soy grits by acid hydrolysis. A number of enzyme hydrolysates are also available as flavoring agents.

Oriental soy foods, both fermented and nonfermented products, are part of the daily diet in many areas of the world. Products such as soy sauce (shoyu), tofu, tempeh, and others are becoming more popular in the United States and Europe. Preparation and uses of these soy foods are as follows:

Type	Preparation	Uses
Soy Milk	Aqueous extract of the whole soybean.	Same as cow's milk.
Tofu (soy curd)	Made by coagulation of soy milk. Tofu curd contains 88% moisture, 6% protein, and 3.5% oil. Tofu can also be frozen, aged and dried (56% protein).	Same as milk and cheese. Fresh dried (kori) tofu has a shelf life of six to 12 months.
Tempeh	Composed of cooked soybeans fermented by the mold *Rhizopus oryzae* (protein content about 20% on a wet basis and 50% after drying).	Indonesian dish.
Miso (soy paste)	Made by fermentation of cooked soybeans with the mold *Aspergillus oryzae* grown on rice or barley.	Soup base and condiment.
Soy Sauce	Made by fermentation of a combination of soybeans and cereals, usually wheat.	Flavoring agent.
HVP (hydrolyzed vegetable protein)	Acid and/or enzyme hydrolysis of soy grits.	Flavoring agent.
Whipping Protein	Partial hydrolysis with enzymes.	Whipped proteins.

Soy flour, derived from ground soybeans, boosts protein, brings moisture to baked goods, and provides the basis for some soymilks and textured vegetable protein. This versatile ingredient improves taste and texture of many common foods and often reduces the fat absorbed in fried foods. The taste of soy flour varies from a "beany" flavor to a sweet and mild flavor, depending on how it is processed.

Soy Flour

IN THE MARKET

Soy flour comes in small bags in the baking or natural foods section of supermarkets. In natural foods markets, health food stores, food cooperatives, and food buying clubs, soy flour is often found in bulk bins. Many customers order soy flour through mail order houses and on-line shopping.

Most stores carry at least one of the three types of soy flour:

- full-fat that contains all the natural oils found in the soybean
- low-fat that contains about 1/3 the amount of fat as full-fat, and
- defatted that contains minimal fat as most of the oil is removed during processing.

RETAIL SUPPLIERS

Soy Flour is commonly added to other food products, but is available retail too.

Dixie USA, Inc – Defatted soy flour (mail order)

GIVE ME FIVE

1. Make a batch of homemade pizza dough and replace one-fourth of the flour with soy flour.
2. Make soy nut butter or peanut butter cookies and replace 1/3 of the all-purpose flour with soy flour.
3. Make antioxidant-rich blueberry muffins and pancakes more whole grain: use 1/3 soy flour, 1/3 whole wheat flour and 1/3 all-purpose flour.
4. Make lemon poppy seed, zucchini, or banana walnut bread and replace 1/3 of the all-purpose flour with soy flour.
5. Bake a nutrient-packed carrot cake with pineapple, raisins and walnuts and use replace 1/3 of the all-purpose flour with soy flour.

IN THE KITCHEN

Storing and Cooking Tips for Soy Flour:

- Kept in an airtight container, defatted and low-fat soy flour will stay fresh for up to one year. Full-fat soy flour will keep for up to one year in an airtight container in the freezer.
- KeptStir soy flour before measuring to avoid flour packing.
- KeptWatch baked goods closely for over-browning. Baking products in a lower temperature oven (less 25° F) may prevent browning.

Full-fat and low-fat soy flours work best in sweet, rich, baked goods like cookies, soft yeast breads and quick breads. In these recipes, soy flour will substitute well for ten to 30 percent of the wheat or rye flour. Recipes specifically developed to use soy flour may replace more than 30 percent of other flours with soy. Replacing more than 40 percent of other flours with soy flour is not recommended because soy-rich dough browns faster. Since soy flour is gluten-free, it cannot replace all the wheat or rye flour in yeast raised bread. Soyfood cookbooks, soy flour packages, and company web sites supply tasty recipes.

NUTRITION HIGHLIGHTS

Soy flour is a great source of high quality soy protein, dietary fiber and important bio-active components, such as isoflavones. This versatile ingredient

provides a good source of iron, B vitamins and potassium. Important bio-active components found naturally in soybeans are being studied in relation to relieving menopausal symptoms, such as hot flashes, maintaining healthy bones, and preventing prostate, breast cancers, and colorectal cancer. The content and profile of bio-active components varies from product to product, depending upon how much soy protein is in the food and how the soy protein is processed.

Soyfoods are a healthy protein source because of the high quality of protein that contains all essential amino acids needed for growth. Soyfoods are a good source of essential fatty acids and contain no cholesterol and little or no saturated fat. This comparison of the protein content of several flours indicates the high protein content of soy flours in relation to wheat flours*:

- Full-fat soy flour 40 % protein
- Low-fat soy flour 52 % protein
- Defatted soy flour 55 % protein
- Whole wheat flour 16 % protein
- Enriched white flour 12 % protein

* approximately

In addition to the excellent nutritional value of soy protein, scientists have found that consumption of soy protein can contribute to reducing the risk of heart disease by lowering blood cholesterol and increasing the flexibility of blood vessels. The FDA has approved a health claim stating that "25 grams of soy protein in a daily diet low in saturated fat and cholesterol can help reduce total and LDL cholesterol that is moderately high to high."

Nutrition Facts 1/4-cup serving of soy flour provides						
	Defatted	% Daily Value	Full Fat1	% Daily Value	Low Fat	% Daily Value
Calories	82		92		82	
Total Fat	0g	0%	4g	6%	1.5g	2%
Saturated Fat	0g	0%	0.5g	3%	0g	0%
Total Carbohydrates	10g	3%	7g	2%	8g	3%
Protein	12g	24%	7g	14%	10g	20%
Cholesterol	0mg	0%	0mg	0%	0mg	0%
Sodium	5mg	0%	3mg	0%	4mg	0%
Dietary Fiber	5g	18%	2g	8%	2g	8%
Calcium	60mg	6%	43mg	4%	41mg	4%
Potassium	596mg	17%	528mg	15%	565mg	16%
Phosphorus	168mg	17%	104mg	10%	130mg	13%
Folate	76mcg	19%	72mcg	18%	90mcg	23%
Source: USDA National Nutrient Database for Standard Reference, Release 17 (2004)						
Average Total Isoflavones	33mg		37 mg		50 mg	
Source: USDA -Iowa State University Database on the Isoflavone Content of Foods, Release 1.3, 2002, USDA Nutrient Data Laboratory Agricultural Research Service						
Exchanges: 2 tbs. = 1 lean meat/ meat substitute						
Based on information from Exchange List for Meal Planning, 2nd edition, 2002.						

THE MAKING OF SOY FLOUR

A wide array of meat alternatives, dairy alternatives, and baked goods use various forms of soy flour. Soy flour is a product of milling soybean flakes that have either retained the soybean's naturally occurring oil to make full fat flour or solvent-extracted the oil to make de-fatted flour. To make low-fat soy flour, a mechanical extractor process removes about 75% of the oil. Newer technologies extract oil from soy flour using high pressure carbon dioxide or other liquids. Full fat and de-fatted flour products appears in enzyme active or toasted forms and in different particle sizes from ultra fine powders (i.e., soy flour) to more coarse soy grits. Further processing soy flour produces dry textured nuggets called textured soy flour.

Related Proceedings Appendix

None